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20. ABSTRACT (Continue on reverse side if necessary and identify by block number)

ioroscopic, plating, and bacteriophage analysis techniques were developed for llowing bacterial survival and bacterial predation in situ in soil. Several w predators were discovered, and their host ranges were studied. The dominant predators were identified. A new form of competition involving growth initiation factors was discovered.

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Final Report

Title: Survival of Microorganisms and Bacterial Predation in Nature

ARO Proposal Number: P-18 755-L

Contract Number: DAAG29-82-K-0055

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Final Report

Statement of Problem:

This study concerned bacterial survival (or destruction) in soil. Of particular interest was the occurrence and activity of non-obligate bacterial predators of bacteria, as relates to both the destruction and survival of the host and predator bacteria. One goal was to detect, isolate, and study bacterial predators of bacteria. A second goal was to detect and study attack of bacterial predator on bacterial predator. A final goal was to detect and study instances of what appeared to be predation but actually were some other form of antagonism or competition.

Results Summary

We devised a technique that we call "indirect phage analysis." This technique allows us to detect and follow predation of one bacterium on another *in situ* in soil. As the predator multiplies in response to the host cells, it in turn is attacked by indigenous bacteriophage in the soil. These phage are specific for their host. They are extracted from the soil, plaqued on respective host cells in the laboratory, and counted. Fluctuation in their numbers thus relates to the activity in soil of the bacterial predator. This technique, along with other techniques, was used in various of the studies to be reported.

Ensifer adhaerens in soil was found to attack *Micrococcus luteus* and *Myxococcus* strain 8, which is also a bacterial predator of bacteria. *E. adhaerens* also attacked strains 34 and C2 which are streptomycete predators of bacteria. Thus, its activity interlocks with other predation sequences. In a sense, it also attacks *Bacillus subtilis* endospores in soil (see later). Although *E. adhaerens* can attack certain other bacteria, it in turn is attacked by another gram negative soil bacterium, strain N-1, which is a

potent bacterial predator in soil. Two other gram negative predators, however, do not attack E. adhaerens.

Strain N-1 is able to attack several bacterial predators, including Agromyces ramosus. We have found no bacterial predator that can attack N-1.

As a pure laboratory culture, A. ramosus can attack a variety of gram positive and negative bacteria, and even the yeast Saccharomyces cerevisiae. Dead cells are not attacked. In situ in soil, however, its host range seems to be more narrow. It possibly has a growth initiation problem in soil. Thus, it was shown to produce and use the Mg^{++} - Ca^{++} related growth initiation factors and inhibitors discussed later.

Myxococcus strain 8 is a bacterial predator, but it has some tendency to autolyze in soil. Also, it in itself, is a host for attack by at least two other bacterial predators. Nevertheless, it survives well in soil by forming myxospores which are not attacked. These myxospores germinate if the proper host cells are present.

Actinomyces humiferus would seem to act as a bacterial predator, because it can kill certain other bacteria. Indirect phage analysis, however, says that it is not a predator. We found that A. humiferus employs a previously unknown form of competition to kill other organisms. A. humiferus, and the other organisms, produce growth innitiation factors (plus inhibitors for them) that activate or marshal calcium and magnesium for use in bringing on growth initiation. However, A. humiferus is much more effective in making use of the factors, so that the other organisms are deprived of these metals. Several organisms are able to make the factors. But also, the factors (and inhibitors) are made by higher forms of life: A. humiferus was used for the detection and assay of these factors. For example, the factors and inhibitors are present in extracts made from various organs of the guinea pig and cow.

The reason for the presence of these activities in higher life forms can only be postulated at present.

A stain was developed that allows microscopic detection, observation, and study of bacterial endospores *in situ* in soil. This stain can also differentiate between dormant spores and those undergoing the initial stages of germination. This stain, and also plate counts of spores that were incubated in soil, were used to study endospore survival in soil. For these counts, the spores were plated on a medium solidified with Gelrite. We found that this solidifying agent was far superior to agar for spores. The endospores of *B. subtilis*, and the endospores and parasporal crystals of *B. thuringiensis*, were shown to survive well in soil. *E. adhaerens* caused a reversible activation of *B. subtilis* spores for germination. Germination did not proceed further, however, and there was little overall effect on the spores or *E. adhaerens*. Nevertheless, although not studied, these activated spores should show increased sensitivity to attack by other soil organisms. Predators N-1 and L2 attack *B. subtilis* spores *in situ* in soil, as was shown by indirect phage analysis. N-1, however, also attacks *E. adhaerens*.

Except under very unusual conditions, *B. thuringiensis* spores do not germinate to any extent in soil. We therefore used *B. thuringiensis* spores to demonstrate the existence of a soil-mediated "sporostasis" in soil. The spores are not harmed by the sporostasis. At present we have evidence both for and against a hypothesis that fungi somehow cause this sporostasis.

Aside from the possibility that fungi can cause bacterial sporostasis, we isolated a soil fungus that had an absolute requirement for bacterial endospores for growth. Unfortunately, this requirement was lost during successive transfers in the laboratory. We also isolated several soil fungi which utilize bacterial spores, but they do not have an obligate requirement for the spores.

An overview of our studies of bacterial predators of bacteria seems to show that the predators can be placed into two groups, roughly corresponding to their gram reactions. The gram positive ones tend to have a narrow prey organism range, have a recognizable dormant form, and require nutritionally enriched soil for detection of predation. They may themselves serve as hosts for the gram negative predators. Some representatives of this group are Agromyces ramosus and Streptomyces species. The gram negative predators have a relatively wide prey organism range, have no known dormant form, and do not require enriched soil. Some of them can attack the gram positive predators. Enisfer adhaerens, as well as strains N-1 and L2 and some others, are representatives of this group. Of all the predators we have looked at, strains N-1 and L2 seem to be dominant. Neither can attack the other, but they do have some host organisms in common.

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